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DOCKET NO.: 15054-3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Ming-Tang Chang, et al.		
SERIAL NUMBER:	09/477,371	GROUP:	1638
FILING DATE:	January 6, 2000	EXAMINER:	C. Collins
TITLE:	**ANIMAL FEED WITH LOW PHYTIC ACID, OIL BURDENED AND PROTEIN LADEN GRAIN**		

Certification Pursuant to 37 CFR § 1.8
I certify that the attached correspondence was transmitted by facsimile to 703 872-9306, on the date set forth below.

Patricia A. McDaniels

Patricia A. McDaniels
Reg. No. 33,194

January 29, 2004

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

In support of the above-identified application, Peter L. Keeling states the following.

1. I received my B.Sc. (Honors) from The Hertfordshire University in Applied Biology in 1976, and my Ph.D. from the Council for National Academic Awards, Surrey University in 1981. From 1981 to 1986 I was a Senior Research Scientist and Biochemistry Group Leader at ICI Corporate Bioscience Laboratory, Starch Biosynthesis Group, in the United Kingdom. From 1986 to 1988 I was the Grain Filling Work Group Leader at Zeneca Seeds Bioscience Research Laboratory in the United Kingdom. From 1988 to 1994 I was Applied Biology Project Leader at the ICI Seeds Bioscience Research Laboratory, Biochemistry, Cytogenetics and Physiology Group in Slater, Iowa. From 1994 to 2001, I was the Director of Research of ExSeed Genetics L.L.C., and I am currently the Unit Director of BASF Plant Science L.L.C. Ames Research Facility. I have also been Associate Professor with the Agronomy Department of Iowa State University. I have been actively leading a team of research scientists in conducting research in plant science, in particular in the field of starch deposition, since 1981. I have been an external

examiner for many M.S. and Ph.D. theses. I have published extensively in the area of plant starch biosynthesis, and am a named inventor on nine U.S. patents. I was actively involved in the development of the NutriDense® grain product, which is characterized by enhanced levels of protein and oil, and which is now sold by BASF Plant Science L.L.C. I am a co-inventor of the present application. I have read and understood the Office Action dated July 29, 2003, and I am familiar with the Declaration Pursuant to 37 C.F.R. § 1.132 submitted on April 22, 2003 (hereinafter the "First Declaration").

2. The data presented in the First Declaration, and the data presented in the present Declaration relate to hybrid corn produced using inbreds which are owned by germplasm "originators". Originators allow their inbreds to be sold to make hybrids, under the condition that the source of the inbred is kept confidential. Accordingly, BASF Plant Science GmbH, the assignee of the present application, is obligated contractually not to disclose the sources of inbreds used to practice the present invention, even though the inbreds are commercially available. In light of these contractual obligations, I can only disclose the number of different originators represented in the data, and state that germplasm proprietary to each originator is generally not closely related to germplasm proprietary to other originators.

3. For the purposes of this Declaration, lines are numbered A1 through A8, B10, C11 through C14, D15 through D17, E73, F18 through F19, G20, H21, I22, J23, L24, M25 through M29, N30, and O31 through O32. Thus lines from 14 different originators are represented in the data presented in this Declaration. For each mutant, there are lower case letters (py or phy) indicating that the particular line is a low phytate mutant. After the lower case letter is a number, indicating the mutational event. A different number indicates a different mutational event. Generally a different mutational event will be a different point mutation. Table 1 of this Declaration shows generally how one of ordinary skill in the art of corn breeding designates a crossing block, and how the crossing blocks presented herein should be read.

TABLE
1

	Male Line 1			Male Line 2		
	Phytate	Protein	Oil	Phytate	Protein	Oil
Female Line 1 Female Line 2 Female Line 3 Female Line 4	Values of Trait in grain from selfed hybrid			Values of Trait in grain from selfed hybrid		

It should be noted that the male and female lines in the crossing blocks set forth herein may be NutriDense® or yellow dent, and that hybrids will exhibit the NutriDense® enhanced protein and oil profiles if either the male or female parent exhibits the NutriDense® protein and oil profiles.

4. Table 2 of this Declaration re-presents the data from Table 1 of the First Declaration in the crossing block format. The data presented in Table 1 of the First Declaration represents seven inbred lines from five different originators, designated originators A through D and F. All protein, oil and phytate (or inorganic phosphorus) contents described in the data presented herein represent the mean values of several measurements of grain and seed. As with all measurements there is some individual variability between samples even of the same genetic materials. In order to minimize this experimental variability, the data presented herein were obtained from multiple seed and grain samples and have errors values in the range of 15-30% of the mean.

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TABLE 2

		Males														
		A5py0380			A1py1656			A1py1672			A1py2148			(A5 x A1)		
		Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil
	A4py0719	54.0	12.3	5.1	22.0	12.8	5.1	11.0	12.9	5.0	16.0	12.0	4.6			
	B10py510	63.0	12.5	5.2				24.0	12.3	5.4	24.0	13.2	5.0			
	C11py138	83.0	12.9	5.0	53.0	14.1	5.1									
	D17py662	66.0	12.0	5.0	35.0	12.6	5.4									
	D17py478															
	F18py170				47.4	12.9	4.9									
Females	A3py1857				25.1	12.2	5.4	10.0	12.8	4.9						

		Males														
		A4py719			B10py510			C11py138			D17py662			D17py478		
		Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil
	A5py0380															
	A1py1656				29.0	12.3	5.4				29.0	12.2	5.9	26.0	12.0	5.8
	A1py1672															
	A1py2148													27.0	12.0	5.7
Females	(A5 x A1)							68.0	12.9	5.0						

Phytate data presented as percent of wild-type

Protein & oil data presented as percent dry weight of seed.

5. Table 3 of this Declaration presents the protein, oil, and phytic acid content of 24 independent low phytate events in comparison with their respective isolines. Protein and oil are presented in Table 3 as percent dry weight of seed, and phytate is presented as percent of wild type. The selection of elite inbred lines for conversion to a low-phytate mutant was based on three criteria: (i) high-yield and agronomic performance using replicated field yield trials, which are standard practice throughout the industry, (ii) ability to combine well with one another when in hybrid combination so as to achieve the highest possible yields, and (iii) being wide-ranging in protein and oil content so as to allow selected crosses to be made so as to produce NutriDense® hybrids. This third criteria is important in the current invention as it creates an F1 hybrid which when grown in a farmer's field will produce NutriDense® Low Phytate grain having a low phytate and protein and oil content which falls between those of the inbred parents.

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TABLE 3

	Pedigree	Yrs of data	Wild Type			LP Mutant			DIFFERENCE (LP-Wild)		
			Phytic Acid (% of wild)	Protein % dry wt	Oil % of dry wt	Phytic Acid (% of wild)	Protein % dry wt	Oil % of dry wt	Phytic Acid (% reduction)	Protein (% reduction)	Oil (% reduction)
Female and Male Inbreds converted to LP Mutants	A1py1212	1	100.0	17.09	5.38	11.8	16.2	5.6	-88.20	-0.91	0.26
	A1py1656	2	100.0	17.09	5.38	28.5	17.1	5.3	-71.46	0.03	-0.05
	A1py1672	2	100.0	17.09	5.38	10.1	16.3	6.1	-89.89	-0.81	0.70
	A1py2148	2	100.0	17.09	5.38	10.9	16.7	5.0	-89.11	-0.37	-0.39
	A1py2236	2	100.0	17.09	5.38	6.7	16.8	5.1	-93.28	-0.29	-0.27
	A2py292	2	100.0	13.73	3.13	25.7	14.4	3.3	-74.27	0.70	0.20
	A3py1104	2	100.0	12.87	4.79	19.3	12.7	5.0	-80.69	-0.17	0.16
	A3py1511	2	100.0	12.87	4.79	45.5	13.1	4.9	-54.49	0.23	0.10
	A3py1857	2	100.0	12.87	4.79	8.8	12.7	4.4	-91.22	-0.20	-0.42
	A4py0719	2	100.0	13.6	4.3	14.4	13.5	4.1	-85.64	-0.01	-0.25
	A4py1232	2	100.0	13.6	4.3	11.1	14.7	3.6	-88.87	1.19	-0.69
	A6-E665phy	1	100.0	12.4	5.0	83.9	14.1	4.8	-16.12	1.67	-0.22
	A7py3645	2	100.0	14.06	4.18	45.2	14.1	3.7	-54.80	0.05	-0.49
	B10py510	2	100.0	13.71	5.28	33.2	14.0	4.4	-66.81	0.25	-0.87
	C11py138	1	100.0	13.70	4.53	79.1	15.6	4.4	-20.91	1.90	-0.13
	C12py834	1	100.0	11.7	4.3	29.3	13.0	4.4	-70.71	1.28	0.17
	C13py620	1	100.0	12.9	4.0	51.3	14.5	3.5	-48.69	1.62	-0.47
	C14py75	2	100.0	13.20	4.46	29.2	14.0	3.8	-70.84	0.76	-0.62
	D15-E167phy	1	100.0	11.48	3.28	82.6	11.8	3.4	-17.42	0.32	0.12
	D16-E674phy	1	100.0	12.37	3.67	70.7	12.7	3.7	-29.29	0.37	0.00
	D17py478	1	100.0	12.67	3.68	60.0	13.2	3.8	-40.01	0.53	0.12
	F18py102	1	100.0	12.0	4.1	46.7	11.0	3.4	-53.33	-0.98	-0.73
	F18py170	1	100.0	12.0	4.1	67.3	12.0	3.7	-32.70	0.05	-0.47
	F19-E36phy	1	100.0	12.42	4.02	45.9	13.7	4.3	-54.11	1.32	0.28
	AVERAGE		100.00	13.73	4.49	38.21	14.08	4.32	-61.79	0.35	-0.17

6. Table 4 of this Declaration presents the inorganic phosphorus, protein, and oil contents for hybrids produced during the 2003 summer growing season. The inbreds used to produce these hybrids were from 9 different originators. Phosphorus (P_i), protein and oil are presented as percent dry weight of seed. The P_i content is inversely correlated with phytate content. Values for wild type lines are shown in bold.

7. The person of ordinary skill relevant to the present invention is a corn breeder, who is familiar with a large variety of inbred lines and their characteristics. Such an ordinarily skilled person would know and understand that protein and oil contents of lines varies relatively little in Elite yellow-dent corn. They would further know and understand that selecting for high-oil and protein and high-yield to create elite NutriDense® inbred lines is challenging but technically feasible, as evidenced by the commercial availability of some such hybrids in the marketplace. Prior to the present application, the person of ordinary skill would not have known that phytate content can be decreased independently of protein and oil content and that elite hybrid NutriDense® Low-Phytate lines are a practical feasibility.

All statements made herein of declarant's knowledge are true, and all statements made on declarant's information and belief are believed to be true. The statements made herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 29th January 2004

Peter L. Keeling

USSN 09/477,371
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TABLE 4

		Males																		
		A5			A5py0300			A5py0731			A1			A1py1656			A1/A1py1658		
		PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil
Females	A8	0.006	11.30	5.20															
	A8py0491				0.084	10.83	5.22	0.237	12.49	4.87				0.178	11.29	5.4			
	A4	0.009	11.64	4.75							0.004	12.12	5.13	0.036	11.23	5.5			
	A4/A4py0719				0.090	10.89	5.02							0.186	12.14	5.2	0.066	11.64	5.41
	A4py0719				0.143	11.79	4.81	0.272	11.62	4.29				0.294	11.69	5.1			
	L24	0.000	10.88	4.57							0.010	11.88	4.85						
	L24/L24py0490				0.042	10.76	5.06							0.082	11.91	5.4			
	L24py0171				0.118	12.10	4.42	0.276	11.60	4.29				0.210	11.94	4.7			
	G20	0.000	10.12	5.01							0.000	11.85	5.23	0.004	11.49	5.8			
	C11	0.003	12.18	5.07							0.003	11.78	5.39						
	C11/C11py0138				0.016	12.44	4.87							0.129	12.76	5.3			
	C11py0138				0.067	11.68	4.91	0.132	12.62	4.87				0.123	11.85	5.3			
	D17	0.009	10.49	4.56							0.011	11.39	4.79						
	D17py0151													0.176	11.72	4.31			
	D17py0478				0.112	11.10	4.60							0.202	11.38	4.8			

		Males																		
		M26py1656			M27py1656			M28py1656			M28py1656			A1py1656/A5py0380			A5py0481		
		PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil
Females	A4py0719/A8py0481													0.198	12.37	5.1			
	A4py0719	0.246	11.79	4.98	0.237	12.03	4.60	0.240	11.20	4.58									
	L24py0171	0.267	11.76	4.69	0.239	12.33	4.67	0.211	12.00	4.44	0.226	12.06	4.78						
	C11py0138	0.143	11.86	4.93	0.150	12.27	5.32	0.128	12.07	4.95	0.120	12.09	5.36				0.054	11.87	4.83

		Males								
		A4			A4py0719			C11py0138		
		Phytate	Protein	Oil	Phytate	Protein	Oil	Phytate	Protein	Oil
Females	A1/A5	0.008	11.61	5.12	0.076	11.68	5.08			
	A1/A5py0380				0.116	11.31	5.02			
	A1/A1py1656				0.133	11.67	5.22			
	A1py1656/A5py0380				0.130	11.74	6.04	0.156	11.29	5.21
	A5/A5py0380				0.127	11.34	4.84			
	A5/A1py1656				0.076	11.31	4.83			

		Males																		
		I22			I22py1049			A619			A619ipa1			M617			M617ipa1		
		PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil
Females	G20	0.020	11.72	3.87															
	N30	0.012	10.31	4.21															
	A8py0491				0.154	11.02	4.89												
	A632							-0.002	10.90	4.43									
	A632ipa1										0.173	11.42	4.28						
	E73													0.002	11.06	4.06			
	E73ipa1																0.177	10.33	4.03

		Males																		
		O31			O32			Pioneer 33P66			Pioneer 34M94			Pioneer 35Y54			Pioneer 36N70		
		PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil	PI	Protein	Oil
Females	G20	0.007	11.18	4.47	0.007	10.02	4.59												
	Pioneer 33P66							0.014	10.56	4.27									
	Pioneer 34M94										0.005	11.24	3.99						
	Pioneer 35Y54													0.002	9.79	4.25			
	Pioneer 36N70																0.012	11.02	4.23